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Chapter 8

Emerging Targeted Therapies for Treatment of Hepatocellular Carcinoma (HCC)

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http://dx.doi.org/10.5772/intechopen.71480

Abstract

Hepatocellular carcinoma (HCC) has dismal diagnosis due to the presence of underlying cirrhosis, late diagnosis, and limited treatment options. Surgery or liver transplantation is restricted to those with small tumours or well-compensated liver diseases. Despite advances in early screening and diagnosis of HCC, survival of patients has not improved greatly. Furthermore, treatment options for advanced HCC are restricted to best supportive care. Currently, sorafenib is the only drug approved for the treatment of advanced HCC patients as well as for those not suitable for transarterial chemoembolization (TACE). Therefore, there is an urgent need to develop new agents for treatment. Hepatocarcinogenesis is a complex multistep process that involves deregulation of various signalling pathways. Thus, there is no dominant molecular mechanism in HCC and understanding of these pathways provides an opportunity for development of potential therapeutic agents in an effort to reverse, prevent or delay tumourigenesis. This review will summarise the significance of these pathways in HCC and discuss the therapeutic benefits or drawbacks of the potential target agents against these pathways especially those that have been part of clinical trials.

Keywords: hepatocellular carcinoma, targeted therapy, sorafenib, signalling pathways, immunotherapeutics

1. Introduction

Hepatocellular carcinoma (HCC) is the most common primary malignancy of liver cancer and is the second biggest cause of cancer-related deaths world-wide. The incidence of HCC is increasing all over the world but the highest rates of HCC are reported in South-East Asia with the leading rate of mortality occurring in China [1]. The risk factors for HCC are well

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defined, such as hepatitis B virus (HBV) infection, hepatitis C virus (HCV) infection, alcohol consumption, and non-alcoholic steatohepatitis (NASH) [2].

The main reasons for the high rate of mortality are lack of diagnostic methods and limited treatment options for patients with advanced HCC. Surveillance programmes to identify patients with early HCC, such as by ultrasound sound screening and by serum alpha fetoprotein (AFP) levels, are not well implemented. Additionally, AFP levels are also dysregulated in benign liver diseases [3]. Some of the treatment options for HCC patients include surgical resection, liver transplantation (LTx), radiofrequency ablation (RFA), transarterial chemoembolization (TACE) and sorafenib. Surgery has a 5-year survival rate of 70% but unfortunately at the time of diagnosis, only 10–30% of patients are suitable for this option. The biggest risk post-surgery is that of recurrence. The 5-year recurrence rate in patients with early HCC is about 68% after surgery [4]. LTx is suggested to HCC patients with tumours within the Milan criteria (a single lesion ≤5 cm, or up to three lesions ≤3 cm each) and is associated with a 5-year overall survival (OS) rate of 75%. However, the limitation of LTx is shortage of organ donation [5]. RFA is another option for patients with early HCC (<3 cm) and its survival benefits are comparable to those with surgical resection. However, high costs of RFA and complications involving peritoneal bleeding hinder its use [6]. TACE is the standard of care for intermediate HCC with preserved liver function, and with no signs of macrovascular invasion or extrahepatic spread. It has reported a median survival of 34 months and a survival benefit at 1, 3, 5 and 7 year as 82%, 47%, 26% and 16%, respectively. However, TACE is a heterogeneous operating technique with variation in efficacy depending on the choice of chemotherapeutic agents used [7]. For advanced HCC patients, sorafenib is the only drug approved by the

Trial name and no.	Drug	Target	Ref
SHARP, NCT00105443	Sorafenib *versus* placebo	VEGFR, PDGFR, Raf	[8]
Asia Pacific, NCT00492752	Sorafenib *versus* placebo	VEGFR, PDGFR, Raf	[15]
STORM, NCT00692770	Sorafenib *versus* placebo	VEGFR, PDGFR, Raf	[17]
REACH, NCT01140347	Ramucirumab *versus* placebo	VEGFR-2	[23]
--, NCT00699374	Sunitinib *versus* sorafenib	VEGFR, PDGFR	[26]
LIGHT, NCT01009593	Linifanib *versus* sorafenib	VEGFR and PDGFR	[28]
RESOURCE, NCT01774344	Regorafenib *versus* placebo	VEGFR1-3, PDGFR-β, and FGFR-1	[30]
EVOLVE-1, NCT01035229	Everolimus *versus* placebo	mTOR	[39]
SILVER, NCT00355862	Sirolimus *versus* placebo	mTOR	[43]
METIV-HCC, NCT01755767	Tivantinib *versus* placebo	c-MET	[47]
JET-HCC, NCT02029157	Tivantinib *versus* placebo	c-MET	[47]
CELESTIAL, NCT01908426	Cabozantinib *versus* placebo	c-MET and VEGFR, PDGFR	[48]

--: no name assigned; NCT: ClinicalTrials.gov number; VEGFR: vascular endothelial growth factor receptor; PDGFR: platelet-derived growth factor receptor; FGFR: fibroblast growth factor receptor; mTOR: mammalian target of rapamycin.

Table 1. Phase III clinical trials of molecular targets in HCC.

US Food and Drug Administration (FDA) for treatment and although it improves survival compared to placebo in clinical trials it suffers from adverse side-effects and high costs [8]. Unfortunately, with a majority of patients still diagnosed at late stage and with clinical phase III trials failing to improve survival benefits in intermediate and/or advanced HCC, new molecular therapeutics are urgently needed to address the dismal prognosis of HCC. One approach is to identify molecular targets from the several signalling pathways that are dysregulated in HCC. Several phase III trials have been completed to identify potential molecular targets in HCC (**Table 1**).

2. Vascular endothelial growth factor (VEGF) receptor signalling

Angiogenesis is a critical step for tumour growth and metastasis. With HCC being a highly vascular tumour, controlling tumour-associated angiogenesis offers a promising approach to inhibiting tumour progression.

VEGF is the most well documented growth factor in angiogenesis. It exerts its effect by binding to its receptors, VEGF receptor 1 (VEGF-R1), VEGF-R2, and VEGF-R3, present on endothelial cells. VEGF secreted by tumour cells bind to its receptors and results in activation of signal transduction pathways promoting cell migration, proliferation, and survival of cancer cells leading to angiogenesis. VEGF overexpression is possibly induced by the hypoxic tumour environment, activation by epidermal growth factor (EGF) receptor (EGFR) and cyloonxygenase-2 signalling. Increased levels of VEGF, VEGFRs have been reported in HCC cell lines, tissue and serum of HCC patients. High levels of VEGF in HCC patients has been associated to poor OS and disease-free survival [9], vascular invasion [10] and portal vein emboli [11]. Additionally, Guo et al. [12] and colleagues reported poor prognosis for HCC patients with increased serum VEGF following TACE. VEGF is also more commonly expressed in HCV-associated HCC than in HBV-associated HCC providing clinical implications for different population of HCCs.

Other growth factors stimulating angiogenesis include fibroblast growth factor receptor (FGFR), and platelet-derived growth factor receptor (PDGFR). Overexpression of either of these growth factors has also been associated to poor survival. There are four types of FGFRs (FGFR1, 2, 3, 4) and the PDGFR consist of PDGFRα and PDGFRβ [13, 14].

Sorafenib is currently the only drug approved for treatment of advanced HCC patients who cannot undergo TACE treatment. It is an orally active anti-angiogenic multi-kinase inhibitor. Several clinical studies have reported promising results. In the randomised phase III SHARP trial (ClinicalTrials.gov number NCT00105443), 400 mg of sorafenib twice daily, significantly increased the OS of advanced HCC patients (7.9 months *versus* 10.7 months) and the time to progression (TTP) (2.8 months *versus* 5.5 months) compared to the placebo group [8]. Similarly in another phase III Asia Pacific trial (NCT00492752), sorafenib increased the OS and TTP from 4.2 months to 6.5 months and from 1.4 months to 2.8 months, respectively [15]. The difference in the OS and TTP results in both studies could be due to patient HCC aetiology. The Asia Pacific trail had more HBV-associated HCC compared to the SHARP trial (73% *versus* 12%).

The use of sorafenib as an adjuvant after surgery or TACE remains doubtful. In a small retrospective study with 36 HCC patients, 12 patients received sorafenib post-surgery and the remaining 24 patients had surgery only. The group of patients who received sorafenib post-surgery had a significantly longer OS (37 months *versus* 30 months) and TTP (29 months *versus* 22 months) [16]. However, in the phase III placebo-controlled study (STORM, NCT00692770), which recruited 1602 patients from 28 countries, sorafenib as an adjuvant treatment after surgery/local ablation, did not affect time to recurrence or OS [17]. Similar findings were reported in the SPACE trial (NCT00855218). In this phase II trial Lencioni et al. [18] tested the efficacy of doxorubicin-eluting beads (DEB)-TACE plus sorafenib *versus* sorafenib in patients with intermediate HCC. The authors did not report a significant improvement in TTP following addition of sorafenib to DEB-TACE.

Bevacizumab is an anti-VEGF monoclonal antibody that has demonstrated improved efficacy in patients with unresectable HCC. Treatment with bevacizumab at 5–10 mg/kg produced partial response (PR) in 14% and disease control rates (DCR) in 56% of patients. A phase II trial of bevacizumab with capecitabine and oxaliplatin (chemotherapeutic drugs) also showed encouraging results with a median progression-free survival (PFS) of 6.8 months, and a median OS of 9.8 months. Twenty three patients had stable disease with overall 77.5% disease control rate and eight patients produced (PR) [19]. Hsu et al. [20] investigated the combination of bevacizumab plus capecitabine in a phase II study yielding median PFS and OS of 2.7 and 5.9 months, respectively.

Ramucirumab is another example of monoclonal antibody targeting VEGFR-2. The above mentioned bevacizumab targets the proangiogenic factor VEGF while ramucirumab blocks the receptor. In a phase II study involving advanced HCC patients, ramucirumab monotherapy yielded a disease control rate (DCR) of 50%, PFS of 4.0 months and OS of 12 months [21]. The promising results from this study lead to a phase III trial (REACH, NCT01140347) of ramucirumab monotherapy in advanced HCC patients (post-sorafenib). Although ramucirumab did not improve OS, interestingly in a sub-group of HCC patients with AFP base line levels ≥400 ng/mL, ramucirumab significantly enhanced the OS [22].

Sunitinib is an orally administered multi-kinase inhibitor with activity against various kinases including VEGFR and PDGFR. It has been approved for treatment of renal cell carcinoma (RCC), and imatinib-resistant gastrointestinal stromal tumours (GIST). However, it is not considered for HCC patients due to its high toxicity. In two phase II studies of sunitinib, 50 mg daily of sunitinib orally, 4 weeks on and 2 weeks off, both Barone et al. [23] and Faivre et al. [24] reported high toxicity. Barone et al. [23] observed treatment-related deaths in 18% of patients and with PR in 12% of patients. Median TTP was 2.8 months and median OS was 5.8 months. Faivre et al. [24] reported 10% deaths related to treatment and 80% patients experienced grade 3/4 adverse effects because of which the study could not proceed to the second phase and was terminated [23, 24]. Similarly, a phase III trial (NCT00699374) comparing sunitinib to sorafenib was discontinued. Patients were administered 37.5 mg of sunitinib once daily or 400 mg of sorafenib twice a day but a majority of patients experienced adverse effects such as thrombocytopenia and neutropenia. Additionally, sunitinib did not show a better OS than sorafenib [25].

Linifanib is a multikinase inhibitor targeting VEGFR and PDGFR. In a phase II trial involving 44 HCC patients with unresectable or metastatic HCC, linifanib yielded a median OS of 9.7 months (compared to 10.4 months in patients with Child-Pugh class A hepatic function) [26]. In an open-label phase III trial (LIGHT, NCT01009593), Cainap et al. [27] compared linifanib with sorafenib treatment in advanced HCC. Both drugs had similar OS with 9.1 months for linifanib and 9.8 months for sorafenib. The median TTP was found to be 5.4 months and 4.0 months for linifanib and sorafenib respectively. However, linifanib caused more adverse side effects than sorafenib, implying sorafenib could be more safe than linifanib.

Regorafenib is a novel diphenylurea multikinase inhibiting VEGFR1-3, PDGFR-β, and FGFR-1. It has been approved for treatment of metastatic colorectal cancer after failure of oxaliplatin and irinotecan-based systemic chemotherapy and has also been approved for treatment of metastatic gastrointestinal stroma tumours after failure of imatinib and sunitinib. This year regorafenib was approved by the FDA as a second-line treatment for HCC. HCC patients not responding to sorafenib now have an option of FDA-approved regorafenib as a second line of treatment. This makes regorafenib the first FDA approved drug for treatment of liver cancer in almost a decade. In a small phase II study involving 36 advanced HCC patients who had progressed following sorafenib, regorafenib at 160 mg once daily in cycles of 3 weeks yielded a median TTP of 4.3 months median OS of 13.8 months. The side effects of regorafenib appeared similar to that of sorafenib such as fatigue, diarrhoea, hypertension and, hand–foot skin reaction [28]. A phase III trial of regorafenib (RESOURCE, NCT01774344) involving 573 patients from 21 countries evaluated the efficacy and safety of regorafenib in HCC patients and observed disease progression after systemic first-line treatment with sorafenib. Regorafenib treatment resulted in a survival benefit of 2.8 months compared to placebo (10.6 months *versus* 7.8 months). The median PSF for patients taking regorafenib was 3.1 months compared to 1.5 months for patients taking placebo. The overall response rate was 11% compared to 4% of patients taking placebo [29]. Following these promising results from the RESOURCE trail, regorafenib was approved by the FDA in April 2017 for the treatment of HCC patients who have previously been treated with sorafenib.

3. RAF/MEK/ERK pathway

The mitogen-activated protein kinase (MAPK) cascade consists of serine/threonine kinases, which converts extracellular molecules such as growth factors, hormones, and differentiation factors, into intracellular signals for regulating several cellular processes including proliferation, apoptosis and migration. The four core proteins kinases of this pathway include, Ras, Raf, MEK and ERK. The pathway is activated by binding of ligand to receptor tyrosine kinases (RTK). In the nucleus, phosphorylation of these four protein kinases regulates gene transcription. Around 58% of HCC cases have activated MAPK pathway with Ras, MEK, ERK and MAPK up regulated in 33%, 40%, 50% and 50% of HCC patients, respectively [30]. This pathway has also been shown to be activated by hepatitis virus infections. Dysregulation of this pathway by hepatitis B virus X protein has contributed to loss of function of the tumour

suppressor p53 [31]. HCV infection has led to anti-apoptotic effect also following activation by Ras/Raf/Mek/Erk signalling [32].

Selumetinib is an oral MEK inhibitor. In a small phase II study (NCT00604721) involving 19 HCC patients with advanced HCC, selumetinib was given at a dose of 100 mg twice per day but the study was terminated at interim analysis because there was no response and the TTP was only 8 weeks. However western blot of biopsy samples taken pre and post treatment showed phosphorylation of MEK1/2, and ERK1/2, suggesting failure of selumetinib was not due to lack of target inhibition [33]. A recent phase I study (NCT01029418) looked into the safety, maximum tolerated dose (MTD), and tolerability of selumetinib in combination with sorafenib in 27 Asian patients with advanced HCC. The MTD of selumetinib was at 75 mg daily with sorafenib at 400 mg twice daily. Common treatment-related adverse events included diarrhoea, rash, and hypertension, fatigue, anorexia and hand-foot and mouth disease. Seven patients had a PR and stable disease for more than 6 months. The OS was 14.4 months. Due to the acceptable adverse events, this combination of selumetinib and sorafenib deserves further evaluation [34].

Another MEK inhibitor, refametinib, was evaluated in a phase II study (NCT01204177) in combination with sorafenib in 95 patients with unresectable HCC. Patients received twice-daily refametinib at 50 mg plus twice-daily sorafenib at 200 mg (morning)/400 mg (evening), with dose escalation to sorafenib 400 mg twice daily after cycle 2. The TTP was 122 days and OS was 290 days. Interestingly, the best responders to the combination treatment were those harbouring Ras mutation. A recently completed proof of concept phase II trial (NCT01915602) of refametinib in combination with sorafenib in Ras mutant HCC has recently been completed with results expected soon. Given that Ras mutations are only observed in 3–5% of HCC patients, this study raises questions about feasibility and costs of screening large cohort of patients to identify a small sub-group with particular mutations.

4. Mammalian target of rapamycin (mTOR) signalling pathway

This pathway is a critical regulator of numerous physiological processes and also plays a pivotal role in cell proliferation and metastasis of transformed human cancers including HCC. It is upregulated in around 40% of HCC and has been associated to poor prognosis and early recurrence independent of underlying liver aetiology [35]. Two mTOR inhibitors have been studied in clinical trials.

Preclinical studies have shown everolimus (taken orally) to dose-dependently inhibit tumour growth in patient-derived xenograft models of advanced HCC [36]. In a phase I/II study (NCT00516165) in advanced HCC patients, Zhu et al. [37] reported daily dose of 10 mg per day to be well tolerated in 28 patients producing a medium PFS and OS of 3.8 months and 8.4 months respectively. The subsequent phase II study involving 28 patients with prior systemic therapy with daily dose of 10 mg could not be completed as two patients remained progression free for 24 weeks. Although everolimus was well tolerated this study had some limitations including small sample size and lack of randomised control. The efficacy of

everolimus was next investigated in advanced HCC patients who did not respond to sorafenib. In a phase III, randomised, double-blind study (EVOLVE-1, NCT01035229) everolimus did not show improvement in OS (7.6 months with everolimus, 7.3 months with placebo). In a separate phase II study (NCT01005199), patients with advanced HCC were compared to those administered sorafenib alone (800 mg) or with everolimus (5 mg) [38]. The results were not encouraging and combination of sorafenib with everolimus did not improve efficacy compared to sorafenib alone with median PFS (6.6 months *versus* 5.7 months), TTP (7.6 months *versus* 6.3 months), and OS (10 months *versus* 12 months) were similar in the Sorafenib alone group *versus* sorafenib + everolimus, respectively. However, loss of tuberous sclerosis complex 2 (TSC2) in HCC has been reported to be predictive of response to everolimus in HCC patients [39]. Immuohistochemical analysis of HCC samples collected in the EVOLVE-1 clinical trial (NCT01035229) had no detection of TSC2 and longer OS than compared to placebo. A larger study is needed to validate the potential of everolimus before it can be used to stratify HCC patients for response to everolimus.

Another mTOR inhibitor, temsirolimus (taken intravenously) has not improved survival either alone or in combination with either sorafenib [40] or bevacizumab [41]. In a recently concluded phase III study (SILVER, NCT00355862), another mTOR inhibitor, sirolimus, did not improve recurrence-free survival (RFS) or OS beyond 5 years in Ltx recipients with HCC but it did improve RFS and OS within 3–5 years. This may suggest the potential use of sirolimus for selection of immunosuppression in LTx recipients with HCC [42].

5. c-MET inhibitors

c-Met is a proto-oncogene that encodes the receptor, MET, for the ligand of hepatocyte growth factor (HGF). MET is a tyrosine kinase receptor regulating metastatic progression. Binding of MET to HGF activates the RAS-MAPK and PI3K-AKT signalling pathways leading to tumour development and metastasis. In HCC, c-MET protein is overexpressed in 70% of HCC and has been associated to poor prognosis [43].

Foretinib was the first c-MET inhibitor of broad spectrum, including c-Met and VEGFR, to be tested in clinical trials. In a phase I/II study (NCT00920192) involving patients with advanced HCC, the median TTP was 4.2 months and the OS was 15.7 months. Its toxicity profile was also acceptable with the most adverse events including hypertension and anorexia. Baseline plasma levels of Interleukin 6 (IL6) and Interleukin 8 (IL8) were identified as independent predictors of OS by multivariate analysis. A larger randomised study is needed to warrant the effects of foretinib [44].

Tivantinib is a selective oral inhibitor of c-MET. In a randomised, placebo controlled phase II study (NCT00988741), advanced HCC patients were administered 240 mg daily resulting in a small improvement in TTP (1.4 months *versus* 1.6 months) compared to the placebo group. Additionally, HCC tumours expressing high levels of c-MET protein, as judged by immunohistochemical analysis, demonstrated an improved OS (7.2 months *versus* 3.8 months) and longer TTP (2.7 months *versus* 1.4 months) compared to placebo. There was no difference

in OS and TTP in HCC patients with c-MET protein expression between tivantinib and placebo. These results suggest the potential of c-met protein expression to select HCC patients who may benefit from tivantinib [45]. However, surprisingly, two large randomised double-blind placebo-controlled phase III trials i.e. METIV-HCC (NCT01755767) and JET-HCC (NCT02029157), have both failed to demonstrate improved OS in advanced HCC patients with high c-met protein expression [46].

Cabozantinib is also an oral inhibitor of c-MET, VEGFR and PDGFR. *In vitro* and *in vivo* studies have demonstrated its reduced invasive and migratory properties in HCC. A phase II randomised trial is on-going to investigate the efficacy to cabozantinib in solid tumours. A phase III, randomised, double-blind, controlled trial is underway to evaluate the efficacy of cabozantinib *versus* placebo as a second-line treatment for advanced HCC who have received prior sorafenib (CELESTIAL, NCT01908426) [47].

6. Other potential therapeutic targets in HCC

6.1. Wnt/β-catenin signalling

The Wnt/β-catenin signalling plays a pivotal role in a host of physiological and pathophysiological processes such as embryonic development, cell proliferation, regeneration, angiogenesis and cancer [48]. It is also an important player in maintaining liver health, but it is found to be dysregulated in HCC with mutation in β-catenin observed in about 40–70% of HCC cases, proving to be a potential important target of therapy.

At physiological levels β-catenin is regulated by a destruction complex consisting of adenomatous polyposis coli (APC)/Axin/glycogen synthase kinase 3b (GSK3β), and casein kinase 1 (CK1) which phosphorylates β-catenin at Ser33, Ser37, Thr41, and Ser45 residues located in exon 3. The phosphorylated β-catenin is polyubiquitinated by β-transducin repeat containing protein (β-TrCP) and degraded by the proteasome. However, wnt signalling is activated upon binding of the wnt to one of the frizzled (FZD) family members and to low- density lipoprotein receptor-related protein 5 (LRP5) or LRP6, resulting in the inhibition of β-catenin degradation. The accumulated cytoplasmic β-catenin translocates to the nucleus where it forms a complex with T-cell factor (TCF)/lymphoid, displacing the transcriptional inhibitor Groucho, and the β-catenin-TCF complex enhances transcription of target genes that are implicated in cancer development for example, c-Myc and cyclin D1.

Nuclear β-catenin accumulation has been found to be associated to tumour progression and poor prognosis. Cytoplasmic β-catenin accumulation has been reported in HCCs larger than 5 cm in diameter and with reduced disease-free survival. Dysregulation of the wnt/β-catenin signalling has also shown to regulate angiogenesis and metastasis [49]. Aberrant activation of wnt signalling has also resulted from deregulation of other components of the pathway e.g. up regulation of wnt genes (Wnt3, Wnt4 and Wnt5A) and FZD (FZD3, FZD6 and FZD7) in about 60–90% of HCCs with more than 5% occurring in peritumours, implying that their expression could be an early event in hepatocarcinogenesis [50].

Disruption of β-catenin and TCF association in the nucleus by two fungal-derived compounds, PKF115-584 and CGC049090, has shown dose-dependent cytotoxicity against HCC cells and 10 times reduced toxicity in normal hepatocytes [51]. The disruption reduced expression of wnt/β-catenin target genes (c-Myc, cyclin D1, survivin) and inhibited *in vivo* tumour growth [52]. For reasons yet to be delineated, the presence of EpCAM, hepatic stem cell marker and a direct target of the wnt/β-catenin pathway, sensitised HCC cells to these antagonists [53]. Together these results suggest that EpCAM expression may facilitate HCC prognosis by effective stratification of HCC patients responsive to wnt/β-catenin signalling antagonists.

Recently, two FDA-approved drugs have been identified to antagonise wnt/β-catenin pathway by differing mechanisms. First, pyrvinium was identified in a chemical screen for small molecules. It binds to CK1 potentiating its activity and leading to stabilisation of the destruction complex resulting in degradation of cytoplasmic and nuclear levels of β-catenin [54]. Recently, Pimozide, an antipsychotic drug, has been shown to inhibit cell proliferation and apoptosis in HCC cell lines by reducing EpCAM and β-catenin [55]. The specific role of these inhibitors has yet to be completely elucidated.

Another class of compounds regulate the wnt/β-catenin pathway by inhibiting tankyrases (TNK1 and TNK2). TNKs destabilise Axin leading to β-catenin stabilisation. Thus, inhibition of TNKs prolongs half-life of Axin preventing β-catenin accumulation. These compounds include XAV939 and WXL-8 and also reduce tumourigenicity *in vivo* [56].

Another therapeutic strategy to regulate the wnt/β-catenin signalling is to block the interaction between wnt ligands and FZD receptors. This has been achieved with monoclonal antibodies or using recombinant soluble fragment of FZD (sFZD). A monoclonal antibody, OMP-18R5, developed using the extracellular domain of FZD7, binds to five FZD receptors and blocks wnt signalling. It inhibits *in vivo* tumour growth and acts synergistically with chemotherapeutic drugs including taxol, irinotecan and gemcitabine [57]. OMP-18RS, is the only potential compound targeting the wnt pathway to make it to clinical phase I trials (NCT01345201) for the treatment of solid tumours and myeloid malignancies, suggesting potential use for HCC treatment.

Sorafenib has also been proposed as a potential wnt modulator, decreasing β-catenin and also expression of liver-specific wnt targets (GLUL, LGR5, and TBX3) in several HCC cell lines accompanied by reduced tumour volume *in vivo* using HepG2 xenografts in nude mice [58].

Several studies have also evaluated the significance of combination therapy for targeting the wnt pathway. A small molecular target, FH535 inhibits proliferation of HCC cell lines by inhibiting recruitment of β-catenin coactivators and also suppresses peroxisome proliferator-activated receptor (PPAR) signalling. Galuppo et al. [59] reported FH535 and sorafenib synergistically inhibited HCC cell line and liver cancer stem cells by targeting the RAS/RAF/MAPK and WNT/β-catenin pathways. Western blot demonstrated cleaved increased poly (ADP-ribose) polymerase (PARP) and reduced cyclin D1 and c-Myc.

Identification of pharmacological inhibitors of the wnt/βcatenin pathway is still underway. In the complex network of wnt ligands, receptors and β-catenin, preclinical studies have yielded promising results but wnt inhibitors targeting HCC have not yet reached clinical trials.

6.2. Immunotherapeutics

Immune checkpoints are emerging as promising targets for treatment of HCC. The immune system helps to distinguish body's own cells from foreign cells. To help it achieve this, immune checkpoints which are molecules on certain immune cells, need to be activated or inactivated to start an immune response. Cancer cells find ways to use such checkpoints to escape immune response.

Programed death-1 (PD-1) is a check-point receptor found on $CD8^+$T-cells and directs it from attacking other cells in the body. PD-1 binds to PD ligands (PDL)-1 and PDL-2. Cancer cells have high amount of PD-1 and PDL-1 which helps them evade immune response. In HCC, $CD8^+$ T-cells that express PD-1 is much higher in both tumour regions and peripheral blood compared to healthy controls [60, 61]. Additionally, several correlative studies have associated PD-1 and PDL-1 in tumours to be significantly associated with HCC recurrence, and poor prognosis [62, 63]. Monoclonal antibodies against PD-1 and PD-L1 have been developed and are under clinical trials in HCC patients.

Cytotoxic T lymphocyte associated antigen 4 (CTLA-4) is another example of an immune check-point, which serves an inhibitory co-receptor that interferes with T cell activation and proliferation. CTLA-4 pathway downregulates an immune response by binding to CD80. Inhibiting the CTLA-4 pathway leads to T-cell activation and proliferation and may help generate memory T cells. Monoclonal antibody against CTLA-4 are also under trial in HCC [64].

Pembrolizumab and nivolumab are monoclonal antibodies targeting PD-1. A phase II study (KEYNOTE-224, NCT02702414) is currently underway to access the toxicity and activity of pembrolizumab in advanced HCC patients who have been treated with sorafenib. The primary objective of this study is to determine the objective response rate (ORR) of pembrolizumab given as monotherapy. Recently, a case study reported a decrease in tumour size and AFP levels in a 75 year old man with metastatic HCC who was treated pembrolizumab after failure to respond to sorafenib [65]. Another single-arm phase II trial of pembrolizumab is underway (Keytruda, NCT02658019) and is recruiting advanced HCC patients with unresectable HCC. The primary end points of this study are PFS, OS, RR, duration of response and toxicity.

A phase I/II study is underway (CheckMate040, NCT01658878) to evaluate the safety and tolerability of nivolumab. HCC patients who were either not responsive to sorafenib or failed sorafenib are also included in the study. Another phase III study (CheckMate-459, NCT02576509) is recruiting HCC patients to compare nivolumab with sorafenib as a first line treatment for advanced HCC patients.

Durvalumab is a monoclonal antibody targeting PDL-1. A phase II study (NCT02519348) is currently recruiting patients with unresectable HCC to evaluate durvalumab and tremelimumab either alone or in combination.

Tremelimumab is an inhibitor of CTLA-4. In a small pilot clinical trial including 21 patients with metastatic HCV-related HCC, tremelimumab induced a significant decrease in viral load

and showed promising partial response rate and disease control rate of 17.6% and 76.4%, respectively. The TTP was 6.5 months [66].

6.3. Epigenetic-based therapeutics

Chromatin remodelling is a critical epigenetic mechanism regulating gene expression and plays an important role in cell proliferation, differentiation and DNA repair. Epigenetics alters gene expression without any changes to the DNA sequence and involves the enzymatic covalent modification of histones such as methylation, phosphorylation and acetylation. Histone deacetylases (HDACs) remove the acetyl group from histones, making DNA more compact resulting in gene silencing. There are a total of 18 HDACs identified in mammals. Accumulating evidence suggests the overexpression of HDACs to be correlated advanced tumour stage, recurrence after surgery and poor prognosis in several cancers including HCC [67, 68]. For these reasons HDAC inhibitors may serve as potential therapeutic targets.

Currently 2 HDAC inhibitors have been approved by the FDA i.e. vorinostat and romidepsin, for treatment of cutaneous T cell lymphoma. These inhibitors demonstrate anti-tumour activity by means of histone hyperacetylation reducing DNA-histone affinity and allowing access to transcription factors enhancing gene expression. Currently, a phase I clinical trial (NCT01075113) is underway to evaluate vorinostat in combination with sorafenib in HCC. Romidepsin has not been tested in a clinical setting.

Aberrant up regulation of several HDACs (HDAC1, 2, 3, 4, 5, and 11) and changes in copy number of HDAC3 and HDAC5 have been reported in HCC. Treatment with panobinostat (HDAC inhibitor) demonstrated strong anti-tumour activity *in vitro* and *in vivo* and the effect was enhanced in combination with sorafenib [69]. In a recently completed phase I/II trial (SHELTER, NCT00943449), combination of resminostat (HDAC inhibitor) with sorafenib yielded a progression-free survival-rate of 12.5% for resminostat alone and 62.5% for resminostat plus sorafenib. Median TTP and OS were 1.8 months and 4.1 months for resminostat and 6.5 months and 8.0 months for the combination, respectively [70]. These results support further evaluation of HDAC inhibitors in clinical settings in HCC.

7. Conclusion

New therapeutic options are needed for the treatment of HCC despite the availability of sorafenib, which has limited survival benefits in advanced HCC patients. Several clinical trials are investigating the efficacy and tolerability of combining sorafenib with other agents. Future studies should continue to delineate dysregulated signalling pathways in hepatocarcinogenesis to introduce new molecular targets for therapeutic intervention. Simultaneously it is critical to identify biomarkers and/or aberrant genotypes that would predict clinical efficacy to these targeted agents. Much work also remains to evaluate the role of targeted therapy in adjuvant, neoadjuvant or metastatic settings to determine the most suitable combination of treatment. The battle against HCC is far from over and requires a multidisciplinary approach.

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